

Effects of Vitamin E and Zinc Supplementation on Antioxidants in Beta thalassemia major Patients

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Abstract

Objective: In beta thalassemic patients, tissue damage occurs due to oxidative stress and it happens because of the accumulation of iron in the body. This study was conducted to determine the effect of zinc and vitamin E supplementation on antioxidant status in beta-thalassemic major patients.

Methods: This double blind randomized clinical trial was carried out on 120 beta thalassemic patients older than 18 years. Patients were randomly categorized in four groups. Zinc (50mg/day) and vitamin E (400mg/day) supplements were administered for former and latter group, respectively. In the third group both supplements were administered in similar doses. The fourth (control) group received no supplement. The effect of supplementations on serum zinc and vitamin E, superoxide dismutase (SOD), glutathione peroxidase (GPX), total antioxidant capacity (TAC) and body mass index (BMI) were measured at the beginning and the end of the study.

Findings: Serum zinc levels in group 1 and 3 were significantly increased ($P<0.007$ and $P<0.005$, respectively). Serum vitamin E levels in group 2 and 3 were also increased significantly ($P<0.001$). Mean GPX activity in group 1, 2 and 3 decreased significantly ($P<0.015$, $P<0.032$ and $P<0.029$, respectively). Mean SOD activity and TAC did not show significant change after supplementation. BMI had significant increase in all treated groups ($P<0.001$).

Conclusion: Our results suggest that beta thalassemic patients have enhanced oxidative stress and administration of selective antioxidants may preclude oxidative damage.

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Key Words: Vitamin E; Zinc; Glutathione Peroxidase; Superoxide Dismutase; Body Mass Index; Antioxidants

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Introduction

Beta-thalassemia major results from acute reduction or lack of synthesis of beta globin chains and can be produced by gene mutations.

This causes much of the production of alpha-globin chains which are not able to produce hemoglobin tetramer^[1]. Nearly 200 million people in the world are affected by thalassemia syndromes^[2]. The prevalence of thalassemia gene in the world is about 3%. About 15,000 people are known as thalassemic in Iran, and about 3,000,000 people are carrying thalassemia gene^[3].

In beta thalassemia major, tissue damage occurs due to oxidative stress, and it happens because of the accumulation of iron in the body. Spontaneous oxidation of unpaired alpha-globin chains leads to production of super oxide ions and hydrogen peroxide^[4-7].

Reduction in the Total Peroxyl Radical Trapping Capacity (TRAP), which indicates decreased antioxidant defense mechanism, is seen in this disease. Toxic oxygen metabolites are scavenged by antioxidant enzymes such as super oxide dismutase (SOD) and glutathione peroxidase (GPX). SOD reacts directly with H₂O₂ and then enzymes (Catalase and GPX) come to practice. Thus, these three enzymes, in addition to specific roles, work together against oxidant factors^[5,8,9]. Vitamin E is the most important fat soluble antioxidant and considered as the primary step for antioxidant defense system. Numerous studies have demonstrated reduced serum level of this vitamin in beta-thalassemic patients^[10-12].

One study conducted on beta-thalassemia intermedia patients indicated that vitamin E supplementation improved balance of the antioxidant system and inhibited the process of lipid peroxidation in patients^[10]. In addition to vitamin E, zinc deficiencies in beta-thalassemia major patients have been reported in some studies^[11,13-20]. Zinc plays several profoundly and biologically important roles such as causing structural effects in more than 300 metalloenzymes like super oxide dismutase. It also plays different antioxidant roles in the body^[21,22]. Specific dietary recommendations for thalassemic patients minimize consumption of iron in the diet, and unfortunately result in the reduction of zinc intake^[23]. Furthermore, chelator factors administered to prevent the accumulation of iron,

cause an excretion effect on zinc in these patients^[7,24,25].

Micronutrient deficiencies, especially of vitamin E and zinc in beta-thalassemia major patients have been reported frequently. However, there is no published data about supplementation of these nutrients in thalassemia major patients.

So, we conducted a trial to investigate the joint and separate effects of vitamin E and zinc supplementation on antioxidant status such as GPX, SOD and total antioxidant capacity [TAC] in beta thalassemia major patients.

Subjects and Methods

This double blind randomized clinical trial was carried out on 120 beta-thalassemic major patients older than 18 years admitted to Taleghani and Amiralmomenin hospitals in the Golestan province (northern Iran) in 2009. All patients were characterized for having beta globin gene mutation.

Inclusion criteria were the history of continuous treatment with blood transfusion and Desferal as a chelator agent. Exclusion criteria were diabetes mellitus, smoking, hepatitis B and C and consumption of any supplements at least three months before the study.

The patients were randomly assigned into four groups including 30 subjects in each group using a random allocation method (random allocation method was a random permuted block or block balanced randomization using 8 characters blocks containing A, B, C and D). The first and second groups were asked to take a supplement of 220 mg zinc sulfate containing 50 mg zinc daily for three months and 400 mg vitamin E supplement daily for three months. The third group took both supplements in similar doses. The fourth (control) group received no supplement.

Information about the demographic characteristics was obtained by questionnaire. Weight and height of subjects were measured. The body mass index (BMI) was calculated as weight [kg]/height [m].

Blood samples were obtained from subjects after a 12-hour fasting, just before they received transfusion at beginning and end of the study, and

stored at -70°C until assay. Vitamin E and zinc therapy in thalassemic patients started immediately after the first transfusion.

Serum vitamin E level was measured by HPLC (Knuer Germany)^[7]. In order to determine serum zinc concentration, the samples were collected in trace metal-free tubes to avoid contamination. Serum zinc was analyzed by flame atomic absorption spectrophotometry (Chem's tech analytical UK, 2000-A.A.S)^[26].

In order to assess the enzyme activity of SOD and GPX, the whole blood was rinsed three times with 0.9% saline normal. Activities of GPX and SOD of RBC were assessed by RANCEL and RANSOL (RANDOX, UK) kits. TAC was assessed by TAC (RANDOX, UK) kits.

The ethical committee of Tabriz University of Medical Sciences approved the study protocol. Written informed consent was obtained from all patients.

Statistical analysis was carried out on Statistical Package for Social Sciences (SPSS for Windows, release 11.0, 2002, SPSS, Chicago, IL, USA). Data were presented as mean \pm SD. Paired t-test was used for comparison of the mean for changes of different variables before and after intervention phases. Significant level was set 0.05 and all the statistical tests were carried out as two sided.

Findings

One hundred and twenty subjects including 57 males and 63 females participated in this study.

The mean (\pm SD) age for all groups was 21.1 (\pm 9.7) years. Males' mean \pm SD age was 21.6 \pm 3.8 and for females it was 22.2 \pm 4.6 years.

Serum zinc concentration in group 1 and 3, was significantly increased ($P<0.007$ and $P<0.005$, respectively). Serum concentration of vitamin E, in group 2 and 3 was significantly increased ($P<0.001$). Mean GPX activity in groups 1, 2, and 3 showed significant decrease ($P<0.015$, $P<0.032$ and $P<0.029$, respectively).

Mean BMI in groups 1, 2, and 3 revealed significantly positive changes ($P<0.001$).

Mean SOD activity and serum TAC did not show significant changes in any of the studied groups.

No significant difference was seen in fourth (control) group, which did not use any supplements (Table 1).

Discussion

Red blood cells in thalassemics have morphologic abnormalities, which result in increased susceptibility of thalassemic red cells to the exogenous peroxidant threat. Two mechanisms for inadequate peroxidant defense in thalassemics are insufficient vitamin E levels in red blood cells and plasma, and decreased activity of several enzymes including SOD and GPX, which are the first line of defense against oxidant stress^[7].

According to the results (Table 1), mean serum levels of vitamin E in all groups at beginning of the study were lower than the normal range^[27].

Vitamin E deficiency in thalassaemias is attributed to its increased consumption pursuant to the oxidative stress^[5,7]; Chronic hepatic iron overloads, while causing a substantial reduction of serum lipids, can also lead to concurrent reduction of serum vitamin E^[28]. Some studies have reported a serum zinc deficiency in beta thalassemia patients, which was related to hyperzincuria resulted from the release of Zn from hemolyzed red cells^[18,19,20].

In our studied subjects, 16% had a serum zinc deficiency (data are not shown); however, mean serum zinc was not low. Similarly, Mehdizadeh et al also did not report a zinc deficiency in beta thalassemic patients^[29].

Absence of predominant zinc deficiency in our patients may have resulted from regular transfusion therapy.

Our findings indicate that supplementation with Zn, vitamin E or both of these causes a significant increase in serum level of zinc in the first group, increased serum vitamin E in the second group, and increased serum zinc and vitamin E levels in the third group with no significant difference in the control group. Therefore, these interventions, especially vitamin E supplementation had favorable effects on serum vitamin E levels in patients, considering that base level of vitamin E in serum of all studied groups was lower than the normal limit.

Table 1: Mean (SD) of variables before and after interventions

Parameter	G1		G2		G3		G4		
	before	after	P value	before	P value	before	P value	before	P value
Zn ($\mu\text{g/dl}$)	84.6 (14.8)	163.7 (14.5)	0.007*	132 (192.4)	101.2 (32.7)	0.4	89.5 (23.1)	238.4 (256.1)	0.005*
Vit. E (mg/dl)	0.2 (0.1)	0.3 (0.1)	0.5	.3 (0.2)	0.9 (0.5)	0.001*	0.3 (0.2)	.8 (0.5)	0.001*
SOD (U/grHbg)	1723.8 (719.8)	1744.8 (620.4)	0.7	1924.8 (631.1)	1759.5 (569.7)	0.5	1839.9 (577.9)	1808.7 (610.4)	0.8
GPX (U/gr Hbg)	46.8 (8.1)	41.9 (6.6)	0.02*	46.1 (6.4)	38.9 (14.7)	0.03*	46.6 (5.9)	42.3 (6.3)	0.03*
TAC(mmol/L)	1.2 (0.2)	1.1 (0.2)	0.2	1.2 (0.2)	1.1 (0.3)	0.4	1.1 (0.2)	1.1 (0.3)	0.7
BMI(kg/m ²)	19.3 (2.6)	20.1 (2.7)	0.001*	19.8 (2.2)	20.3 (2.3)	0.001*	19.6 (2.1)	20.8 (2.2)	0.001*

G1: group 1, or zinc supplemented group.

G2: group 2, or vitamin E supplemented group.

G3: group 3, or zinc and vitamin E supplemented group.

G4: group 4, without supplementation.

* Significant differences between before and after intervention.

Oxidative stress is the result of an imbalance between free radical production and reduced degradation^[30]. An increased oxidant stress and a decreased antioxidant status promote peroxidative damage to cell and organelle membranes. It is well documented that disturbances of oxidant-antioxidant balance occur in hemoglobinopathies, especially in thalassemia and sickle cell diseases^[31]. Removal of toxic oxygen metabolites is the putative function of antioxidant enzymes such as SOD and GPX. It has already been demonstrated that oxidative stress induces antioxidative enzymes, including SOD and GPX^[5]. Our findings confirm this. As indicated in Table 1, prior to interventions, SOD and GPX activities in all groups were much higher than in those of healthy subjects^[31,32]. Significant increase in catalytic activities of SOD and GPX also were found in beta-thalassemic erythrocytes of beta-thalassemic carriers in the study of Filiz et al^[31].

The increased activity of SOD in beta thalassemia may be involved in scavenging the superoxide radical (O_2^-), thereby producing more hydrogen peroxide in the erythrocytes.

The increased activity of GPX in beta-thalassemia may be involved in detoxifying hydroxyl radical (OH^-). This finding suggests that high iron produces an oxidative stress in cells, which respond by increasing their antioxidant defenses. The increase of intracellular antioxidant enzymes might be hypothesized to be a direct effect of increased intracellular iron on gene expression^[5].

Based on our results (Table 1), SOD and GPX activities in subjects were also higher than normal limits at the end of the study. However, GPX activity showed a significant reduction in all treated groups. Therefore, all three types of interventions had protective effects on this enzyme, and were effective in elevating the antioxidant status. These findings confirm the results of other studies^[31,32]. Pfeifer et al reported that after vitamin E administration, thalassemic patients presented a significant reduction in levels of erythrocyte RBC-reactive oxygen species (ROS) and serum thiobarbituric acid reactive substances (TBARS), and concluded that it could be useful for reducing oxidative damage in other target organs of beta-thalassemia intermediate patients^[33]. Meiling Cheng et al demonstrated that supplemen-

tation with vitamin E prevents oxidative damage to low-density-lipoprotein (LDL) and erythrocytes in beta thalassemia patients^[34].

In addition to vitamin E, it was established that zinc can reduce the iron-mediated oxidation of lipids (including red blood cells), proteins, and DNA^[35]. The precise antioxidant role of zinc is unclear, but two mechanisms were proposed; first the competitive effect of zinc with iron and copper on the surface of cell membrane for special protein binding which is specific for preventing the production of the toxic OH radical. The second one is binding to the -SH groups in some proteins and preventing them from oxidation^[36]. Sidhu et al found that in zinc and protein deficient rat the activities of GPX, catalase, and peroxidation of lipids were increased^[37]. Our results confirmed antioxidant effect of zinc (Table 1), as seen in zinc supplemented group GPX activity also reduced significantly.

According to the results (Table 1) SOD activity in groups did not change significantly after supplementation. The study of Rahul Naithani, et al in beta-thalassemia major patients showed that SOD increases significantly, and it may be a compensatory mechanism to keep formation of superoxide anions checked to combat the oxidative stress^[32]. Kessab-Chekir, et al observed that iron, ferritin, SOD, GPX and TBARS were increased in 56 beta-thalassemic patients; however, vitamin E and TRAP reduced considerably^[5]. Meral, et al reported that because of increased lipid peroxidation in beta thalassemic patients a compensatory increase will be seen in SOD and GPX activity^[38].

Our findings confirmed the results of above mentioned study, and showed higher SOD activities in patients. Similar to our findings, Carpino et al indicated despite the increased activity of SOD in transfusion dependent thalassemics, TAC did not change^[39].

Dissayabutra et al reported despite vitamin E and vitamin C supplementation in beta-thalassemia major patients, TAC did not change during the study period^[40]. It was suggested that various methods, including TAC, developed to measure the total antioxidant capacity of serum, have been indicated as useful tools to predict the risk of free radical-induced tissue damage.

Nevertheless, with thalassaemia patients, such

an approach appears unsuitable. Changes in contributors such as uric acid and bilirubin, the levels of which increase in thalassaemia because of hemolysis, may mask marked changes in other essential antioxidants^[28]. It seems that these conditions also contributed to unchanged SOD activity and TAC in our subjects.

Low BMI in beta-thalassemic patients is common^[41]. According to findings (Table 1) BMI of all treated subjects increased significantly, which was indicative of well-being of patients and positive effect of supplementation. Similar results were seen in Das et al study^[12].

It should be noticed that, we did not evaluate other components of the cellular antioxidant defense system such as catalase, glutathione, uric acid, bilirubin, vitamin C, etc. If more enzymatic and non enzymatic antioxidants were measured, we could have a complete evaluation of the antioxidant status in these patients. So, they are considered as limitations of our study.

Conclusion

Our findings indicated that beta-thalassemic patients were under enhanced oxidative stress condition, and vitamin E deficiency. Administration of selective antioxidants revealed helpful effects, especially on serum vitamin E and GPX activity as components of antioxidant system, and on general well-being of the subjects. Further studies are suggested with different dose and duration of antioxidant supplements to determine their benefits in beta thalassemic patients.

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Conflict of Interest: None

References

1. Weatherall Dj. The Thalassemias: Disorders of Globine Synthesis. In: Alichtman M, Beutler E, Kipps TJ, et al. Williams Hematology, 7th ed. New York: McGraw Hill, 2006; Pp: 633-66.
2. Kwiatkowski J. Hemoglobinopathies. In: Lanzkowsky P. Pediatric Hematology and Oncology, 3rd ed. Amsterdam: Academic press, 2000; Pp:124-44.
3. The statistical report of the office of programming for the prevention of the Beta thalassemia major in Iran, The health and medical education and genetic office of the ministry of health and medical education, Iran, 2001; P: 28.
4. Scott MD. H₂O₂ injury in beta-thalassaemic erythrocytes: Protective role of catalase and the prooxidant effect of GSH. *Free Radical Biol Med* 2006;40(7):1264-72.
5. Kessab Chekir A, Laradi S, Ferchichi S, et al. Oxidant, antioxidant status and metabolic data in patients with beta thalassaemia. *Clin Chem Acta* 2003;338(1-2):79-86.
6. Chakraborty I, Mitra S, Gachhui R, et al. Non-haem iron-mediated oxidative stress in haemoglobin E beta-thalassaemia. *Ann Acad Med Singapore* 2010;39(1):13-6.
7. Dhawan V, Kumar KhR, Marwaha RK, et al. Antioxidant status in children with homozygous thalassaemia. *Indian Pediatr* 2005;42(11):1141-5.
8. Chan AC, Chow CK, Chiu D. Interaction of antioxidants and their implication in genetic anemia. *Proc Soc Biol Med* 1999;222(3):274-82.
9. Michiels C, Raes M, Toussaint O, et al. Importance of Se-GPX, Catalase and Cu/Zn-SOD for cell survival against oxidative stress. *Free Radical Biol Med* 1994;17(3):235-48.
10. Tesoriere L, D'Apra, DAllegra M, et al. Oral supplements of vitamin E improve measures of oxidative stress in plasma and reduce oxidative damage to LDL and erythrocytes in beta-thalassaemia intermedia patients. *Free Radic Res* 2001;34(5):529-40.
11. Nasr MR, Ali S, Elgabry E. Antioxidant micronutrients in children with thalassaemia in Egypt. *East Mediterr Health J* 2002;8(4-5):490-5.

12. Das N, Das Chowdhury T, Chattopadhyay A, et al. Attenuation of oxidative stress-induced changes in thalassemic erythrocytes by vitamin E. *Pol Pharmacol* 2004;56(1):85-96.
13. Arcasoy A, Canata D, Sinav B, et al. Serum zinc levels and zinc binding capacity in thalassaemia. *J Trace Elem Med Biol* 2001;15(2-3):85 -7.
14. Firky SI, Saleh SA, Sarkis NN, et al. Study of nutritional status among thalassaemia patients in Damanhour medical national institute. *J Egypt public Health Assoc* 2003;78(1-2):73- 93.
15. Kajanachumpol S, Tatu T, Sasanakul W, et al. Zinc and copper status of thalassemic children. *Southeast Asian J Trop Med Public Health* 1997; 28(4):877-80.
16. Vatanavicharn S, Prigsulka P, Pringsulka P, et al. Zinc and copper status in beta-thalassemic/Hb-E disease and Hb-H disease. *Acta Haematol* 1982; 68(4):317-20.
17. Arcasoy A, Cavdar A, Cin S, et al. Effects of zinc supplementation on linear growth in beta-thalassaemia (a new approach). *Am J Hematol* 1987; 24(2):127-36.
18. Al-Samarrai AH, Adaay MH, Al-Tikriti KA, et al. Evaluation of some essential element levels in thalassaemia major patients in Mosul district, Iraq. *Saudi Med J* 2008; 29(1):94-7.
19. Moafi A, Mobaraki G, Taheri SS, et al. Zinc in thalassemic patients and its relation with depression. *Biol Trace Elem Res* 2008;123(1-3): 8-13.
20. Bekheirnia MR, Shamshirsaz AA, Kamgar M, et al. Serum zinc and its relation to bone mineral density in beta-thalassemic adolescents. *Biol Trace Elem Res* 2004;97(3):215-24.
21. Reily C. The nutritional trace metals, 2nd ed. India: Black Well Publication. 2006; Pp: 82-116.
22. Powell S.R. The antioxidant properties of zinc. *J Nutr* 2000;130(5S Suppl):1447s-1454s.
23. Al-Refaie FN, Wonke B, Wickens DG, et al. Zinc concentration in patients with iron overload receiving oral iron chelator 1,2-di methyl-3-hydroxypyridin-4-one or desferrioxamine. *J Clin Pathology* 1994;47(7):657-60.
24. Aydinhok Y, Coker C, Karacli K, et al. Urinary zinc excretion and zinc status of patients with beta thalassaemia major. *Biol Trace Elem Res*. 1999; 70(2):165-72.
25. Hoffbrand VA. Deferioprone therapy for transfusional iron overload. *Best Pract Res Clin Haematol* 2005;18(2):299-317.
26. Amani R, Saeidi S, Nazari Z, et al. Correlation between dietary zinc intakes and its serum levels with depression scales in young female students. *Biol Trace Elem Res* 2010;137(2):150-8.
27. Mahan LK, Escott-Stump S. Krause's Food and Nutrition Therapy. 12th ed. Philadelphia: Saunders. 2008; Appendix, P: 1235.
28. Livrea MA, Tesoriere L, Pintaudi AM, et al. Oxidative stress and antioxidant status in beta-thalassemia major: iron overload and depletion of lipid-soluble antioxidants. *Blood* 1996;88(9): 3608-14.
29. Mehdizadeh M, Zamani G, Tabatabaee S. Zinc status in patients with major beta-thalassaemia. *Pediatr Hematol Oncol* 2008; 25(1):49-54.
30. Vaculin S, Franek M, Vejrazka M. Role of oxidative stress in animal model of visceral pain. *Neurosci Lett* 2010;477(2):82-5.
31. Şimşek F, Öztürk G, Kemahli S, et al. Oxidant and antioxidant status in beta thalassaemia major patients. *J Ankara Univ Faculty Med* 2005;58(1): 34-38.
32. Naithani R, Chandra J, Bhattacharjee J, et al. Peroxidative stress and antioxidant enzymes in children with beta-thalassaemia major. *Pediatr Blood Cancer* 2005;46(7):780-5.
33. Pfeifer WP, Degasperi GR, Almeida MT, et al. Vitamin E supplementation reduces oxidative stress in beta thalassaemia intermedia. *Acta Haematol* 2008;120(4):225-31.
34. Cheng ML, Ho HY, Tseng HC, et al. Antioxidant deficit and enhanced susceptibility to oxidative damage in individuals with different forms of alpha-thalassaemia. *Br J Haematol* 2005;128(1): 119-27.
35. Fraga CG, Oteiza PI. Iron toxicity and antioxidant nutrients. *Toxicology*. 2002;180(1):23-32.
36. Bao B, Prasad AS, Beck FW, et al. Zinc supplementation decreases oxidative stress, incidence of infection, and generation of inflammatory cytokines in sickle cell disease patients. *Transl Res* 2008;152(2):67-80.
37. Sidhu P, Garg ML, Dhawan DK. Protective effects of zinc on oxidative stress enzymes in liver of protein deficient rats. *Drug Chem Toxicol* 2005; 28(2):211-30.
38. Meral A, Tuncel P, Sürmen-Gür E, et al. Lipid peroxidation and antioxidant status in beta-thalassaemia. *Pediatr Hematol Oncol* 2000;17(8): 687-93.
39. Carpino A, Tarantino P, Rago V, et al. Antioxidant capacity in seminal plasma of transfusion-dependent beta-thalassemic patients. *Exp Clin Endocrinol Diabetes*. 2004;112(3):131-4.
40. Dissayabutra T, Tosukhowong P, Seksan P. The benefits of vitamin C and vitamin E in children with beta-thalassaemia with high oxidative stress. *J Med Assoc Thai* 2005; 88 Suppl 4:S317-21.
41. De Sanctis V, Vullo C, Katz M, et al. Gonadal function in patients with beta-thalassaemia major. *J Clin Pathol* 1988; 41(2):133-7.